Title: Genetic manipulation of *Cryptococcus curvatus* for tailor-made fatty acid production

Group: Systems and Synthetic Biology

Project type: MSc thesis

Credits: 36

Supervisors: Zeynep Efsun Duman-Ozdamar (MSc), Dr. Maria Suarez-Diez, Emil Wolbert

Examiners: Dr. Maria Suarez-Diez, Dr. Rob Smith

Contact info: efsun.duman@wur.nl, maria.suarezdiez@wur.nl, robert1.smith@wur.nl

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Description:

Type of thesis: Experimental work.

Description:

Fatty acids are often used to increase beneficial health properties (polyunsaturated fatty acids, PUFA's), safety/shelf-life (longer-chain fatty acids, hydroxy-fatty acids), or as a surfactant (oils, hydroxy-fatty acids) of chemical, cosmetics, and personal care products. These oils and fatty acids have been mostly gathered from plants (i.e. palm, coconut, olive, sunflower) however the increasing demand leads to severe consequences on the ecosystem. Oleaginous yeasts have great potential for the renewable production of lipids. *Cryptococcus curvatus* ATCC 20509 (*C. curvatus*) is well-known oil-producing yeasts and able to accumulate oils up to 40-60% of their biomass under nutrient-limited conditions (N-limitation) [1,2]. On the other hand, its natural capacity is limited to develop an economically feasible production process. Therefore, obtaining enhanced lipid accumulation and modifying the fatty acid composition profile systematically is essential. One of the strategies to achieve this is designing and modifying these oleaginous yeasts as cell-factories for enhancing the yield of lipid production and for modifying the fatty acid production profile.

This project starts with engineering *C. curvatus* via CRISPR/Cas9 system based on the investigations on the transcriptional landscape of this organism [3,4]. Afterwards, lipid production and growth profile of these mutated strains will be characterized. Selected strains will be further investigated under different fermentation conditions. Samples will be collected to identify the biomass composition that is obtained via engineered strains and at different fermentation conditions.

References:

[1] A. Patel, D. Karageorgou, E. Rova, P. Katapodis, U. Rova, P. Christakopoulos, L. Matsakas, An overview of potential oleaginous microorganisms and their role in biodiesel and omega-3 fatty acid-based industries, Microorganisms. (2020). https://doi.org/10.3390/microorganisms8030434.

[2] N. Pham, M. Reijnders, M. Suarez-Diez, B. Nijsse, J. Springer, G. Eggink, P.J. Schaap, Genome-scale metabolic modeling underscores the potential of Cutaneotrichosporon oleaginosus ATCC 20509 as a cell factory for biofuel production, Biotechnol. Biofuels. 14 (2021) 1–17. https://doi.org/10.1186/s13068-020-01838-1.

[3] F. Bracharz, T. Beukhout, N. Mehlmer, T. Brück, Opportunities and challenges in the development of Cutaneotrichosporon oleaginosus ATCC 20509 as a new cell factory for custom tailored microbial oils, Microb. Cell Fact. 16 (2017) 1–15. https://doi.org/10.1186/s12934-017-0791-9.

[4] L. Wong, B. Holdridge, J. Engel, P. Xu, Genetic tools for streamlined and accelerated pathway engineering in Yarrowia lipolytica, in: Methods Mol. Biol., 2019: pp. 155–177. https://doi.org/10.1007/978-1-4939-9142-6_11.

Used skills:

- Advanced molecular biology techniques (CRISPR/Cas9)
- Biochemistry and analytic techniques
- Genome scale metabolic models.

Requirements:

- Basic microbiology (cell culture, transformation)
- Molecular biology (PCR design, cloning)